EXHIBIT 4

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF PENNSYLVANIA

IN RE: ZOLOFT (SERTRALINE HYDROCHLORIDE) PRODUCTS LIABILITY LITIGATION	:	**MDL NO. 2342** **12-MD-2342**
	:	**HON. CYNTHIA M. RUFE**
THIS DOCUMENT RELATES TO: ALL ACTIONS	:	

PLAINTIFF STEERING COMMITTEE'S GENERIC REPORT
OF T.W. SADLER, Ph.D

July 16, 2013

Generic Report of T.W. Sadler, Ph.D

I have been retained by Plaintiff Steering Committee counsel to render an expert opinion regarding: (1) the role that serotonin (5-HT) plays in embryonic development of certain organs; (2) how maternal exposure to selective serotonin reuptake inhibitors (SSRIs) like Zoloft (sertraline) can affect embryonic development; and (3) whether Zoloft can and does cause or substantially contribute to congenital birth defects including laterality, cardiac, craniofacial, neural tube, limb, ventral body wall and genitourinary malformations.

I. Qualifications

I am an embryologist/developmental biologist/ teratologist who has worked in the field for over 40 years. I received a Ph.D. in Anatomy and Embryology from the University of Virginia in 1976. Between 1976 and 1982, I taught Anatomy and Cell Biology, first at the University of Virginia School of Medicine and then the University of Cincinnati College of Medicine. From 1982-2002 I taught the same subjects at the University of North Carolina in Chapel Hill.

I conducted research in embryology and the origin of birth defects from 1982 to 2002 at the University of North Carolina, Chapel Hill where I became a Full Professor in 1988. I was Co-Chair and Founder of the North Carolina Folic Acid Council and Co-Chair and Founder of the Council for Prevention of Neural Tube Defects in North Carolina, which established a Folic Acid Program and Active Birth Defects Registry in North Carolina. I also Founded and Directed the Birth Defects Center at the University of North Carolina in 1992.

During this time, I was one of the first scientists to study the role that serotonin plays in fetal development including as a regulator of craniofacial morphogenesis and in cardiac morphogenesis. In addition to the published studies my colleagues and I presented the papers at several scientific meetings.

My professional experience includes being Editor of the journal *Teratology* and a member of the Human Embryology and Development Study Section at the National Institutes of Health. In 2002, I received the Godfrey P. Oakley, Jr. Award from the National Birth Defects Prevention Network for my significant contributions to the field of birth defects.

I am an expert in embryology. I have over 200 related publications, including articles, abstracts and books. I am the author of the medical textbook, *Langman's Medical Embryology,*

which is in its 12th edition and has been translated into 12 different languages and is used throughout the United States and the world.

Currently, I am an Adjunct Professor of Pediatrics at the University of Utah, Visiting Professor of Cell Biology and Anatomy at the Quillen College of Medicine at East Tennessee State University, Senior Scholar at the Greenwood Genetics Center in Greenwood, SC and a Consultant in Embryology and Birth Defects Prevention, giving lectures and assisting states with their birth defect prevention campaigns.

II. Summary of Opinions

Serotonin (5-HT) is an ancient and essential signaling molecule, involved in cell to cell communication in many organisms across all reaches of the animal phyla, from flatworms to fruit flies, to sea urchins, and ultimately all vertebrates and mammals, including man (Lauder, 1993; Azmitia, 2001; Buznikov et al., 2001). As a signaling molecule, 5-HT regulates fundamental developmental phenomena, including cell proliferation, migration, differentiation, and gene expression. In turn, these key processes play essential roles in embryological development. Signaling is regulated by concentrations of 5-HT, and by which 5-HT receptor(s) are activated. There are 7 distinct families and at least 15 subpopulations of 5-HT receptors that are coupled to important signaling pathways. This large number of receptors allows 5-HT to control a diverse array of crucial cellular functions which regulate many aspects of normal embryological development.

Selective serotonin reuptake inhibitors (SSRIs), including Zoloft (sertaline), alter cellular concentration levels of serotonin. As a result, signaling pathways under the influence of 5-HT, that are essential for normal embryonic development, are disrupted thereby causing a variety of birth defects.

One of the most sensitive stages of embryonic development to the teratogenic effects of SSRIs like Zoloft occurs very early, during the second week of gestation, when the embryo is patterned with regard to its left-right (L-R) and anterior-posterior (A-P; head-to-tail) axes. At this early stage, 5-HT is utilized as a signaling molecule to establish the genetic pathway necessary for appropriate L-R patterning. If this signaling is disrupted, as happens with Zoloft and other SSRIs during gestational exposure, a variety of birth defects can be induced, including virtually every type of heart malformation, neural tube and craniofacial defects, limb abnormalities, and malformations of the ventral body wall and genitourinary systems.

Organ system development also depends upon 5-HT signaling at later times in gestation. During

organ/tissue differentiation, cell signaling perturbation caused by SSRIs like Zoloft results in abnormalities to specific embryonic structures. During heart development, altered 5-HT signaling adversely affects neural crest cell development, and/or crest cell migration and proliferation, resulting in congenital heart defects. Neural tube defects occur when 5-HT signaling is disrupted in cells involved in formation of the neural folds. Limbs are adversely affected when abnormal signaling perturbs chondrogenesis. Ventral body wall/gut malformations may be induced when signaling through the laterality pathway is altered. Craniofacial birth defects (face and skull) are caused by adverse effects on neural crest cells responsible for formation of these structures. In each of these malformations, 5-HT concentrations are critical for maintaining normal cell signaling such that, when these concentrations are altered by SSRIs like Zoloft, congenital birth defects, as stated above, result.

In summary, it is my opinion, stated to a reasonable degree of scientific certainty, that maternal ingestion of Zoloft, especially during the first trimester of gestation, disrupts serotonin concentrations and cell signaling, which are critical to normal development of the embryo, resulting in birth defects to multiple organ systems, including, cardiac, nervous, craniofacial, skeletal, gastrointestinal and genitourinary. The foundation for my opinions stated herein is based upon my background, training and experience as an embryologist, as well as generally accepted scientifically sound evidence, including but not limited to, research, literature and documents regarding SSRIs and Zoloft, known critical roles of 5-HT in cell signaling and a high degree of biological plausibility for the mechanism of injury, as will be set forth more fully in the body of this report.

III. Teratogens

A teratogen is a chemical, environmental pollutant, pharmaceutical compound, or other toxicant that causes birth defects (Wilson, '77). SSRIs like Zoloft are an example of a class of pharmaceutical compounds that act as teratogens, resulting in a variety of birth defects. Like all teratogens, Zoloft, causes or substantially contributes to birth defects as evidenced by the Principles of Teratology, formulated by one of the pioneers of the science, Dr. James Wilson. Dr. Wilson formulated these principles in 1959 based upon the birth defects disaster caused by the drug thalidomide, which was prescribed to pregnant women as an anti-nauseant. Maternal ingestion of thalidomide caused severe and distinct limb abnormalities in babies exposed to the drug during gestation (McBride, '61; Lenz, '62). The thalidomide tragedy provided a vital lesson because it revealed that drugs could cross the placenta and adversely impact developing fetuses. Dr. Wilson determined that out of thalidomide tragedy, a valuable lesson should be learned:

drugs should not be given "indiscriminately" to mothers during pregnancy (Wilson, '77). They can and do cause birth defects.

According to Wilson's principles, the teratogenicity of a compound, i.e., the type and severity of the defects it causes, is determined by the following 6 parameters:

1. Teratogenesis, abnormal development due to environmental factors, is a result of the interaction of the genetic identity of the fetus with the environment.

2. The fetus is more or less susceptible to certain teratogens at different stages in development.

3. Teratogens act on developing structures and tissues in specific ways.

4. Several factors can affect teratogenesis, such as the duration or amount of exposure to teratogen and the mother's genetic identity.

5. There are four types of teratogenesis: Death, Malformation, Growth Retardation, and Functional Defect.

6. The manifestations enumerated in the fifth principle increase in frequency and severity as the amount of teratogen in the environment increases from a No Observable Adverse Effect Level (NOAEL) to a 100% Lethal Dose (LD100).

In 1972, Dr. Ray Shenefelt authored a seminal paper that expounded on Wilson's important principles of teratology by revealing that birth defects can be induced in the same organ or tissue at multiple stages of an embryo's development (Shenefelt, '10). The study was conducted using the teratogen retinoic acid with very brief exposure times (so that precise estimates of embryonic stages of development could be ascertained) in order to determine the periods of teratogenic susceptibility ("critical periods") of over 72 organs and tissues. In one example, an analysis of palate susceptibility showed that there were four different time frames when cleft palate defects could be induced. As expected, three of these times occurred when the palate anlage (the first identifiable palate cells) could be detected, but one of the periods of susceptibility occurred at a time prior to the appearance of any "palate" cells, when the embryo was in the initial stages of patterning its L-R and A-P axes of development. Indeed, in 25% of the more than 72 malformations that were studied, the "critical period" occurred prior to the appearance of the anlage of that organ (Shenefelt, '10). The fact that organs could pass through multiple stages of sensitivity to teratogens was an important finding, but the fact that their

development could be altered so early in the period of embryogenesis was a revelation in the field of teratology.

It should also be noted that a single teratogen or a misexpression of a single gene can produce malformations in multiple organ systems. For example, in addition to altering cardiac development, many teratogens also cause craniofacial malformations because both the heart and the face are dependent upon neural crest cells for normal embryogenesis (Webster et al., '86). Thus, if a teratogen interferes with neural crest cells, both the heart and face can be adversely affected (Webster et al., '86; Sadler, '12). Similarly, disruptions in gene expression that alter signaling pathways can affect more than one embryonic organ or structure as, for instance, mutations in the gene *TBX5,* which is involved in heart and upper limb development, result in cardiac and upper limb defects (heart-hand syndromes).

Timing for the induction of congential defects is exquisite, such that a teratogen must be present at the requisite moment in development to have an effect. Thus, if a teratogen operates to alter early endocardial cushion induction and formation during the time when 5-HT uptake occurs in cardiac muscle cells, the teratogen must be present when the myocardium regulates this cushion formation. Further, after this critical period, it is irrelevant whether exposure to the teratogen remains present because a cascade of morphogenesis has been set forth and removal of the teratogen will not reverse the effects. Therefore, it is a fundamental principle of teratology that each organ system passes through critical periods of development, and the effects of teratogen exposure during these vital developmental stages can and does induce embryological malformations despite subsequent elimination of teratogen exposure, as was so elegantly shown in Shenefelt's study (Wilson, '77; Shenefelt, '10).

Susceptibility to teratogens may depend upon the genotype of the mother and/or the embryo. For instance, if the mother has a genetic variation that affects her capacity to metabolize a potential teratogen, the embryo will be exposed to a higher concentration of the toxic compound and for a greater duration. Consequently, there is an attendant increase in teratogenicity and inducement of birth defects under such conditions. These types of interactions between the genome and teratogens are called "multi-factorial interactions" (Wilson, '77). Thus, birth defects may be caused solely by genetic abnormalities (15-25%), solely by toxic compounds (10-15%), or, most commonly, by an interaction of the two (multi-factorial causes; 60%).

Another important factor in determining whether a given drug or environmental factor operates as a teratogen is whether it passes from mother to conceptus/embryo by crossing the placenta. During the critical early stages of organogenesis, the placenta is not yet fully developed. At this stage, the embryo is surrounded by the trophoblast, a shell of cells, which are bathed in maternal blood. The trophoblast, a primitive placenta, appears to take up serum factors from the maternal blood and transport them to the embryo. Diffusion is another method of early maternal-fetal transport of serum factors. Thus, at the vital early stages of embryogenesis, the conceptus can be exposed to potential teratogens including Zoloft, via the trophoblast (Sadler, '12). Interestingly, a system of rodent (rat and mouse) embryo culture has been developed that mimics physiological conditions in humans, such that effects of toxins, like Zoloft, alcohol, retinoic acid, and other teratogens, can be studied in controlled conditions (Sadler, '79; Sadler et al., '82; Warner et al., '83; Sadler and Warner, '84). In this system, the rodent visceral yolk sac functions like the trophoblast in human embryos and can be studied to determine if transport of nutrients and other substances, like teratogens, cross the primitive placenta (Warner et al., '83; Jollie, '90). In later stages of development, potential teratogens are passed to the developing embryo via the placenta. Thus, toxic compounds, like Zoloft, can and do pass from the mother to the conceptus at all stages of embryogenesis.

Identification of human teratogens is a multifaceted approach wherein, a number of factors are evaluated. This includes, but is not limed to, obtaining accurate and thorough maternal histories as well as conducting and evaluating epidemiological and animal studies. Animal studies that employ a variety of species are used to test potential toxins and the data are extrapolated to humans. Extrapolating data from animals to humans is more accurate if the mechanism of action of the teratogen is well-defined and one that is fundamental to normal development for a wide-range of species (Wilson, '77).

For example, 5-HT is a basic signaling molecule vital to normal embryogenesis. SSRIs including Zoloft alter 5-HT concentrations which are essential for proper patterning of heart progenitor cells as well and as for neural crest cell development in embryos. Likewise, SSRIs blocking or nullifying serotonin transport sites (SERT) on the surface of embryonic cells prevents those cells from receiving proper serotonin signaling during embryo development. Further, serotonin's role in patterning heart progenitor cells and formation of neural crest cells are fundamental processes that occur in all vertebrate species, and which are processes essential

for normal heart development. Therefore, assessment of the above-referenced factors facilitates accurate determination as to whether drugs like SSRIs including Zoloft are teratogenic. The probability of the determination is further strengthened when birth defects related to the given developmental process seen in animal models are replicated in the human population. In the case of SSRIs like Zoloft, 5-HT's involvement in fundamental processes of embryogenesis and the drug's attendant interference of 5-HT's role with those vital processes is a well-defined mechanism of injury, as observed in robust multi-species animal data. This information makes it scientifically reasonable to extrapolate the data from animal studies to human experience with greater confidence, and to reach the sound conclusion, to a scientific degree of probability that SSRIs like Zoloft cause certain birth defects. This opinion is buttressed in part by epidemiological data (Berard et al., '06; Bar-Oz et al., '07; Louik et al., '07; Diav-Citrin et al., '08; Berard, '09; Pedersen et al., 09; Bakker et al., 10; Kornum et al., '10; Reis and Kallen, '10; Wurst et al., '10; Malm et al., 11) .

However, there are certain limitations to epidemiological studies, including failure to fully detect the true number of babies with birth defects attributable to the teratogen. This occurs because many babies with severe birth defects are spontaneously aborted or electively terminated and, thus, omitted from the data. As illustrative of this point, the risk of spontaneous or elective abortion is low for a baby with cleft lip, and virtually all of these babies would be born and counted. On the other hand, a significant number of babies with severe heart or neural tube defects may be spontaneously aborted or the defect may be diagnosed in utero and the pregnancy electively terminated. Consequently, collection of epidemiological data concerning severe heart or neural tube defects from live born infants will fail to detect the true account of the incidence of heart or neural tube malformations produced by a teratogen.

A similar problem can arise in animal studies where embryos and fetuses with severe defects may be spontaneously aborted (resorbed) and, thereby, omitted in the data representing the incidence of congenital defects. Typically, abortion (resorption) sites can be counted and recorded. However, birth defects responsible for the occurrence of the abortion cannot usually be determined because of tissue deterioration. Also, there may be extensive post-natal death of animal offspring where they have severe congenital malformations. In such a case, detailed necropsies are essential to determine the causes for these deaths, and to accurately identify the birth defects that may have been contributing factors. Therefore, animal and human

epidemiological data may under-represent the true association between a given teratogen and congential birth defects.

One difficulty in identifying human teratogens relates to the nature of birth defects and their occurrence rate. A "common" birth defect is one that arises with an occurrence rate of 1 per 1000 births, for example, neural tube defects. Heart defects are more common with an occurrence rate of 1 per 100 births. Typically, teratogens do not cause dramatic increases in occurrence rates, and as such, it can be difficult to identify whether a new drug or environmental compound as a teratogen. For example, if a drug increases the relative risk for a child to be born with a heart defect by 50%, the rate of heart defects would be 1.5 per 100 births. Therefore, if the drug is causing 1.5 babies per 100 to be born with a heart defect instead of only 1, then in 1000 births there will be 15 babies with the defect compared to the expected number of 10; and in 10,000 births there will be 150 versus 100; and in 100,000 births there will be 1500 instead of 1000. 500 new babies with heart defects is a lot and creates a large emotional burden on 500 families and a significant economic burden on our health care system. Since it has been estimated that as many as 13.4% of pregnant women are using antidepressants (Cooper et al., '07), the total number of affected babies with heart defects is quite high. And heart defects are only one of several malformations for which epidemiological studies have shown an increased relative risk in women using SSRIs.

In summary, identification of human teratogens is not a new or novel process, but rather, employs a well-proven multifaceted and scientific approach that utilizes evaluation of a number of factors, as referenced above.

IV. Signaling Molecules

Another axiom of teratology, illustrated by Shenefelt's seminal study, is that the same teratogen can produce a variety of birth defects. This concept applies to SSRIs including Zoloft. Dr. Shenefelt's study employed the teratogen retinoic acid which was shown to alter many cellular events, including cell death (apoptosis), cell proliferation, extracellular matrix production, and others that can result in birth defects (Shenefelt, '10). Importantly, another phenomenon disrupted by retinoic acid is cell signaling. As our knowledge of genes and molecular biology increases, it is becoming abundantly clear just how important cell signaling and signaling pathways are to normal development. Cells communicate with each other using

signaling molecules that interact with receptors, thereby initiating signaling pathways. In this way, signaling molecules instruct cells to produce certain organs or tissues. For example, signaling molecules called Fibroblast Growth Factors (FGFs) regulate lengthening of the limbs. Another signaling pathway, involving Sonic Hedgehog (SHH), ensures that the thumb and little finger are correctly positioned (Adapted from *Langman's Medical Embryology*, 12th ed.; Benazet and Zeller, '09). Teratogens can perturb normal signaling and disrupt these pathways, which results in a variety of birth defects, depending on the time in development when signaling is altered, and the signaling molecule or its receptor that is targeted.

Interestingly, the same signaling molecule is often employed in the development of multiple tissues and organs. For example, SHH is involved in brain, vertebral, limb, and craniofacial development (Varjosalo and Taiple, '09). Similarly, members of the same family of molecules, such as FGFs, BMPs, TGFβs, WNTs and others are employed in formation of multiple embryonic structures from limbs to the central nervous system. Thus, a single molecule or family of molecules can regulate development of multiple embryonic organs and tissues. In part, receptors for these molecules make this possible. Typically, there are multiple receptors for each signaling molecule, such that the same signal can be interpreted by different cell types, depending upon the receptors that the given cells possess. It is as if a signal molecule contains different codes and how a cell responds depends on which codes can be read by which receptors located on different cells, such that some form limbs, others palate, etc. 5-HT is a critical signaling molecule that is employed in the formation of multiple organs and tissues (Lauder, '93; Azmitia, '01; Buznikov et al., '01). In addition, 5-HT plays a crucial role in establishing the L-R axis and patterning of cells at the earliest stages of embryogenesis (Fukumoto et al., '05a,b; Levin, '05; Levin et al., 06; Vandenberg et al., '13). To regulate these different events, 5-HT utilizes seven families of receptors and fifteen subtypes of these receptors within the families which allows 5-HT to signal multiple events that regulate key developmental processes, such as gene expression, cell proliferation, differentiation, and migration (Azmitia, '01; Kroeze et al., '02).These signaling pathways explain how SSRIs like Zoloft cause a variety of birth defects. As with any signaling molecule, there is an optimum concentration that is required for activation of receptors, such that too much or too little of the signal molecule, in this case 5-HT, disrupts the pathway and triggers abnormal development. Because SSRIs like Zoloft block reuptake of 5-HT at SERT sites, they alter 5-HT intracellular processes that would be initiated by the blocked

serotonin as well as altering extracellular concentrations, either of which result in disruption of 5-HT signaling essential to normal embryogenesis.

The type of birth defect produced by SSRIs like Zoloft is determined by the well-established and widely accepted principles of teratology, as determined by Wilson ('77) and Shenefelt (10). Based upon these principles, one can reasonably state that if a woman ingests SSRIs including Zoloft early in her pregnancy when cells are being patterned (2^{nd}-3^{rd} weeks), multiple and different types of birth defects can be produced. If the drug is taken later in gestation (3^{rd} to 8^{th} weeks), the type of birth defect caused will be determined by the drug's effects on whichever developing organ system is vulnerable at the time. The outcome will also be influenced by dose of the teratogen, duration of exposure, and the other principles of teratology as set forth above by Wilson ('77) and Shenefelt ('10).

V. Establishment of the Body Axes and Patterning in the Early Staged Embryo: Increased Sensitivity to SSRI (Zoloft)-Induced Teratogenesis

In the 2^{nd} and early 3^{rd} weeks of development, the anterior-posterior (A-P; cranio-caudal), left-right (L-R) and dorso-ventral (D-V) axes are being established and the embryo may be most susceptible to teratogenic insults from SSRIs like Zoloft. At this stage, the embryo is in the form of a bilaminar disc, like an oreo cookie without the icing. The anlage of organ systems are not yet present. One of the signaling molecules essential to establishing the L-R axis of the embryo is 5-HT (Fukumoto et al., '05a,b;Vandenberg et al., '13). At this point in embryogenesis, increased concentration of 5-HT on the right side restricts expression of Nodal, another signaling molecule on the right side while decreased concentration of 5-HT on the left side allow Nodal expression on the left side (Fukumoto et al., '05a,b; Levin, '05; Levin et al., '06; Vandenberg et al., '13). This differential localization of these 2 molecules triggers a signaling pathway ending with expression of *PITX2*, essentially, a master gene for left sided development (Fig. 1; Bisgrove et al., 03; Ramsdell, '05). Patterning of the A-P axis is also occurring and the midline is established, in part, by expression of *SHH* (Bisgrove et al., '03; Aw and Levin, '09). During this patterning process, it is essential that both pathways, L-R and A-P (Fig. 1), interact (communicate) to coordinate development of both axes so that organs are placed in the proper position within the body and midline structures, like the neural tube and ventral body wall, develop normally (Bisgrove, et al., '03; Levin, '05; Aw and Levin, '09).

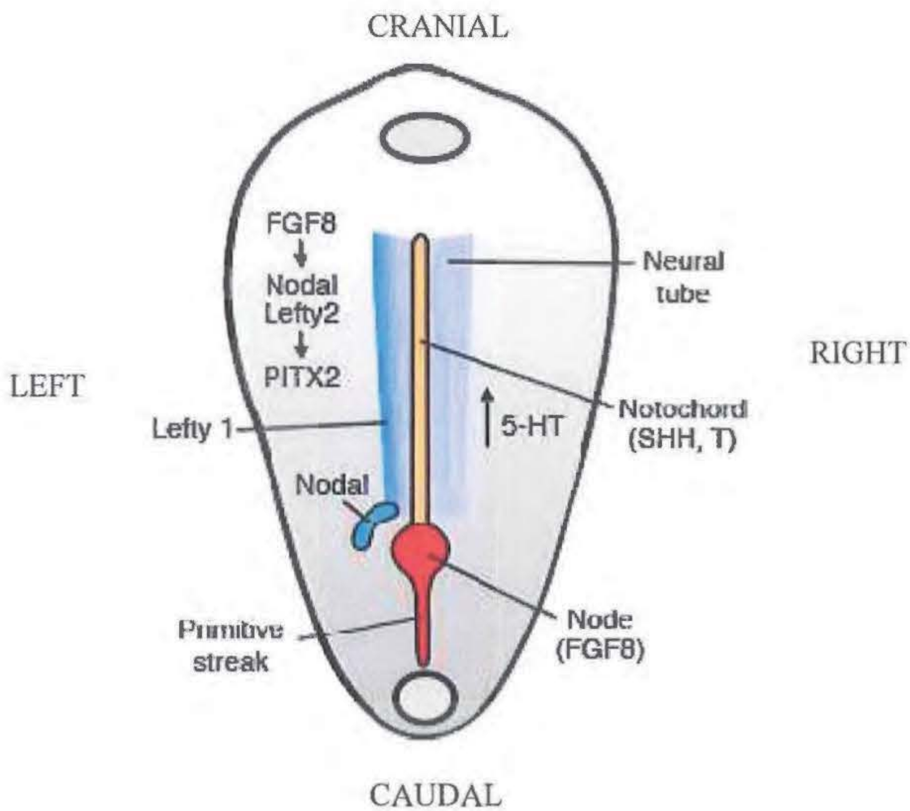

Figure 1. Dorsal (top) view of an embryo at approximately 15 days of gestation showing the establishment of the left-right (L-R) axis. Increased 5-HT concentrations on the right side result in accumulation of the growth factor Nodal on the left, which initiates the signaling cascade ending in *PITX2* expression and delineation of the left side.

Because 5-HT is an important signaling molecule for establishing the L-R axis in embryos, maternal ingestion of SSRIs including Zoloft at this critical early stage of embryogenesis, can disrupt L-R signaling by altering extracellular 5-HT concentrations and interfering with 5-HT entering cells through SERT sites and initiating key intracellular processes. Such a disruption can result in "laterality" defects in the developing embryo/fetus (Fukumoto et al., '05a,b). Normally, many body organs exhibit asymmetry, including the heart, lungs, gut tube, spleen, stomach, liver, and gall bladder. Normal positioning of thoracic and abdominal organs is called *situs solitus*. Complete reversal of all organs, where organs are reversed in a mirror image arrangement, is called *situs inversus*. Discordant organ positioning with respect to symmetry, where one or more organs are abnormally positioned (i.e. reversed in position) or if isomerisms or inversions are present, is called *situs ambiguus* or heterotaxy. These

individuals are considered to have "laterality" defects (Ramsdell,'05) and these defects arise related to a failure to correctly establish left-right patterning during embryogenesis.

Individuals with situs inversus do not have a high risk for having other congenital abnormalities, although they do have an increased risk for congenital heart defects (Ferenz et al., '85; Nugent, 94) and their progeny are at an increased risk of having laterality disease with a greatly increased risk for complex cardiac malformations (Burn, '91; Gebbia et al., 97; Casey, '98). However, individuals with heterotaxy often have other congenital abnormalities, including a variety of midline malformations (Martinez-Frias, '95; Gebbia et al., '97; Kosuki and Casey, '98; Ticho et al., '00; Morelli et al., '01; Bisgrove et al., '03; et al., '04). Furthermore, 90% of these individuals will have complex congenital heart defects (Nugent, '94). Interestingly, within a cohort of individuals with known abnormalities in their laterality signaling pathway, some will have overt heterotaxy or anomalies of organ position, while others will have isolated defects that are often in the midline such as a neural tube defect or omphalocele, or limb abnormalities, such as clubfoot (Morelli et al., '01; Bisgrove et al., '03; Ware et al., '04).

The most sensitive organ to be affected by abnormal L-R patterning is the heart. Virtually every type of heart defect can be observed where there is perturbation in L-R patterning signals, even in the absence of defects to other organs (Ramsdell, '05). Thus, atrial septal defects (ASDs), ventricular septal defects (VSDs), l and d transpositions of the great arteries (TGAs), double outlet right ventricle (DORV), dextrocardia, and many other congenital cardiac defects result from abnormal signaling in the laterality pathway (Dagle et al., '93; Gormley and Nascone-Yoder, '03; Bamforth et al., '04; Ramsdell, '05; Ramsdell et al., '06), as can occur when concentrations of 5-HT are altered by SSRIs like Zoloft (Fukumoto et al., '05a,b; Table 1). Vascular abnormalities, such as aortic arch defects and vena cava defects, as well as total and partial anomalous pulmonary venous return (TAPVR and PAPVR), also arise when abnormal laterality signaling occurs (Van Mierop et al., '72; Rose et al., '75; Heinemann et al., 94; Morelli et al., 01; Ware et al., '04). These types of heart defects are so varied because the heart and blood vessels are dependent upon critical L-R and A-P signaling to acquire their normal development and positioning (Bisgrove et al., '03; Ramsdell, '05). Perturbation of this signaling at the earliest stages of embryogenesis, as caused by prenatal exposure to SSRIs like Zoloft can and does induce a number of complex cardiac malformations, which are considered manifestations of abnormal laterality signaling (Kosaki and Casey, '98).

The link between laterality abnormalities and midline defects has been documented in the clinical literature since at least the mid 1990s. All types of midline defects have been observed in patients with laterality abnormalities, including neural tube defects, cleft lip and palate, gastroschisis, omphalocele, anal atresia and stenosis, and caudal dysgenesis (Martinez-Frias, '95; Gebbia et al., '97; Kosuki and Casey, '98; Ticho et al., '00; Morelli et al., '01; Bisgrove et al., '03; Ware et al., '04). In fact, midline defects so commonly occur when laterality signaling is disrupted that if a patient has a midline defect, it has been estimated that they are 3 times more likely to have a laterality issue as compared to patients without a midline defect, and 100 more times than the general population (Martinez-Frias et al., '95; Morelli et al., '01). Furthermore, because many patients are never assessed for laterality issues, these rates are likely under-reported. More importantly, individuals with midline defects do not require laterality abnormalities to be classified as having disrupted laterality signaling as the primary etiology of their midline malformation. This point has been proven in studies of family members with known mutations to laterality genes. In such families, a high incidence of family members exhibit only a single midline malformation, such as cleft palate or a neural tube defect, with no other abnormality (Morelli et al., '01; Bisgrove et al., '03; Ware et al., 04). Based on these data, one of the criteria for diagnosing problems with laterality signaling and establishing the L-R axis is for a patient to have an isolated midline defect and have family members with heterotaxy (Morelli et al., '01; Bisgrove et al., '03). Therefore, when 5-HT signaling is disrupted during embryogenesis, as results from SSRI ingestion including Zoloft, such that L-R patterning is perturbed, offspring may exhibit isolated heart defects, laterality defects such as asplenia/polysplenia or malpositioned spleen, isolated midline defects, including anencephaly, spina bifida, cleft lip and/or palate, omphalocele, gastroschisis, anal atresia or stenosis, vertebral anomalies, or caudal dysgenesis, or any combination of the above.

Limb defects are also linked to abnormal patterning of the L-R axis. Limb dysplasias and aplasias, as well as clubfoot, have been observed in individuals with heterotaxy (Martinez-Frias et al., '95; Ticho et al., '00; Ware et al., '04). L-R sidedness is observed in limb defects, such that more abnormalities occur in the right limbs than the left (Bod et al., '83; Paulozzi and Lary '99; Gurnett, et al. '08). Furthermore, certain teratogens that preferentially cause limb defects can produce phenotypes that exhibit L-R sidedness. For example, azetozolamide typically causes right sided limb defects in mice. However, when treated mice have situs inversus, i.e., a

laterality defect, the limb abnormalities are randomized with respect to sidedness (Brown et al., '89). Similarly, legless mutant mice exhibit their abnormalities based upon whether they have situs solitus or situs inversus (Schreiner et al., 93). Additional data supporting sideness with respect to limb development and defects is indicated by the fact that children with right sided limb defects have more cognitive deficiencies than those whose malformations are on the left (Dlugosz et al., '88). Clubfoot and other lower limb defects also exhibit sidedness, and some are related to abnormal expression of the laterality gene PITX2, which is regulated by 5-HT at the stage when the L-R axis is specified (Marcil et al., '2003; Gurnett et al., '08). These data support the conclusion that L-R axis patterning is essential for normal limb development, and disruptions in L-R signaling, as caused by altered 5-HT concentrations or SERT blockage, as produced by SSRIs like Zoloft, can cause a variety of limb defects, including limb aplasias, dysplasias, and clubfoot.

Abnormal laterality patterning would also be expected to result in increased pregnancy loss. A tenet of Wilson's principles of teratology holds that the earlier in gestation that an embryo is exposed to a toxic agent the more likely that embryo is to be severely affected and spontaneously aborted (Wilson,'77). Since patterning of the embryonic axes is so important to normal development, and because it occurs so early in gestation, disruptions of this process would be expected to cause severe abnormalities, which in many cases, would result in spontaneous abortion. Such an increase in this type of pregnancy loss has been shown to occur in women taking SSRIs like Zoloft during pregnancy (Baur et al., '10; Broy and Berard, '10; Nakhai-Pour et al., '10; Domar et al., '13).

VI. Heart Development and SSRI (Zoloft)-induced Malformations

As stated under section IV above, which describes the importance of laterality to the origin of many of the birth defects observed in offspring of women taking SSRIs like Zoloft, virtually every type of cardiac defect can occur if 5-HT signaling is disrupted during the establishment of L-R symmetry. In the early period of embryogenesis (14-16 days post-conception), cardiac progenitor cells are forming the Primary Heart Field (PHF), whose patterning is dependent upon proper laterality signaling. Cardiac progenitor cells form during gastrulation as some epiblast cells migrate through the primitive streak and move cranially to a position cranial to the developing neural plate (Fig. 2; Sadler, '12). As they migrate and arrive at

this location, they are patterned from left to right on both sides of the neural plate as to their respective contributions to specific regions of the heart (Abu-Issa and Kirby, '07). This patterning is dependent upon proper signaling through the laterality pathway regulated by 5-HT and, indeed, the heart exhibits more inherent laterality that most other structures, as indicated by its right and left chambers and the origin of its main blood vessels. Consequently, any disruption of this early patterning process, as is caused by Zoloft's and other SSRI's changing extracellular 5-HT concentrations and blocking 5-HT entering cells through SERT sites, alters L-R signaling, which can result in virtually every type of cardiac defect (See Table I at the end Section **VI,** at page 23).

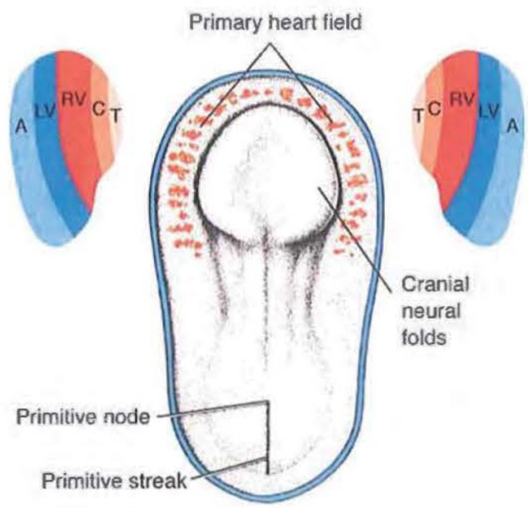

Figure 2. Cells from the epiblast migrate cranially to form the Primary Heart Field (PHF). As they migrate and arrive at their destination, they are patterned on each side into those that will form part of the right ventricle (RV); left ventricle (LV); and Atria (A). The outflow tract consisteing of the conus cordis (C) and truncus areteriosus (T) as well as part of the RV will be formed from the secondary heart field (See text below)

The time frame when laterality and patterning are established is not the only period of susceptibility of the heart to insults by SSRIs like Zoloft. Other stages in heart development are vulnerable because 5-HT acts as an important signaling molecule for critical processes in cardiac differentiation, including: (1) Lengthening the outflow tract via the secondary heart field (SHF); (2) Regulating neural crest cell (NCC) migration, proliferation, and apoptosis; (3) Septating the heart chambers by differentiation of endocardial cushions; and (4) Promoting growth and differentiation of myocardial cells (See Table 1 at page 23).

A. Lengthening the outflow tract via the Secondary Heart Field (SHF) and regulating NCC migration, proliferation and apoptosis

After cardiac progenitor cells are patterned as the Primary Heart Field (PHF) during the 14^{th}-16^{th} days of gestation, they coalesce to form a horseshoe-shaped tube cranial to the neural plate. Then, as lateral body wall folding occurs, the two sides of the horseshoe are brought together in the midline where they fuse to form a single cardiac tube (Fig. 3). The caudal end of the tube represents the atrial region (red), the middle forms the left ventricular region (blue), and the cranial end forms the right ventricle and outflow tract (green and black). During the 23^{rd}-28^{th} days of gestation, looping of the heart tube occurs as the tube bends into the characteristically recognized shape of the heart (Fig. 3). While looping is occurring, the SHF, which forms as a collection of mesenchyme cells ventral to the floor of the pharynx, proliferates and lengthens the outflow tract of the heart tube. If this lengthening does not occur, multiple malformations of the heart and aortic arches are induced, including persistent truncus arteriosus, double outlet right ventricle (DORV), pulmonary stenosis, ventricular septal defects (VSDs), atrial septal defects (ASDs), tetralogy of Fallot, tricuspid atresia, interrupted aortic arch type B, right sided aortic arch, double aortic arch, and retroesophageal right subclavian artery (Yelbuz et al., 2002; Waldo et al., 2005; High et al., 2009). The process involved in lengthening the outflow tract is called convergent extension, whereby cells align themselves with a specific polarity, and it is regulated by the planar cell polarity pathway (PCP; Henderson et al.,'06; Phillips et al., '07; Henderson and Chaudhry, '11). This pathway is also involved in neurulation and gastrulation where it has been shown to be regulated by 5-HT and where disruptions in 5-HT concentrations and signaling cause neural tube defects and caudal dysgenesis (Colas et al., '99a,b; Colas and Schoenwolf, '01; Wang et al., '06; Schaerlinger et al., '07; See Sections **IX** and **X**). Similar disruptions during lengthening of the outflow tract of the heart result in DORV, VSDs, over-riding aorta, and interrupted aortic arch (Henderson et al., '06; Phillips et al., '07).

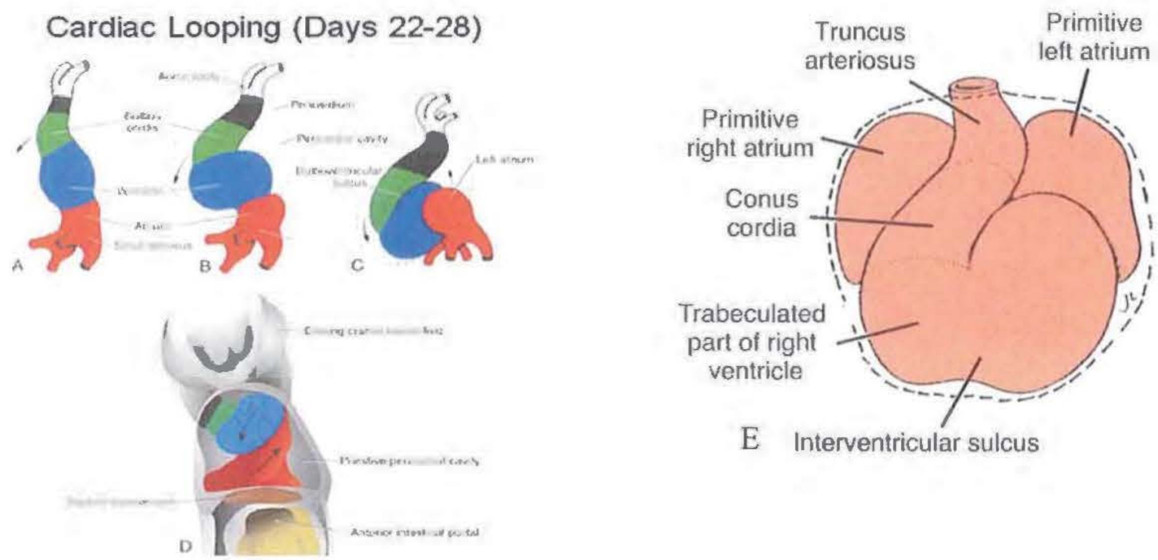

Figure 3. The heart tube is segregated into an atrial region (red), a ventricular region (blue), and an outflow tract region (green and black). Bending of the heart tube (looping; A-D) creates the recognizable shape of the heart (E). Lengthening of the black region of the heart is produced by the secondary heart field (SHF) in concert with cardiac neural crest cells (NCC) migrating to the heart from the cranial neural folds. (See Figure 4 A and B)

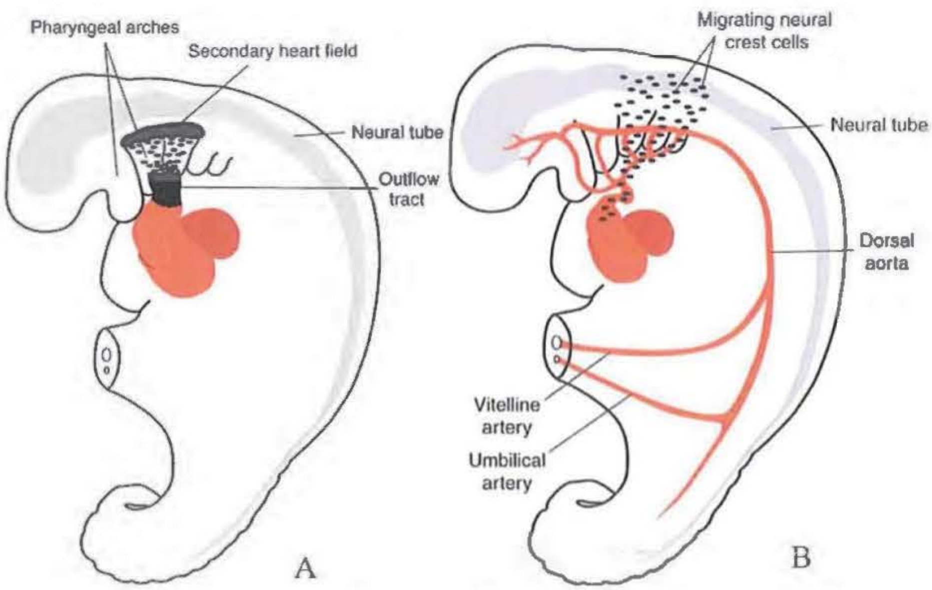

Figure 4. Cells forming in the secondary heart field (SHF) ventral to the pharynx lengthen the outflow tract region (A). Neural crest cells (NCC) migrate in close proximity to cells in the SHF (B) and the 2 cell populations work in concert through interacting signaling pathways to lengthen and septate the outflow tract.

Normal functioning of the SHF requires migrating cardiac neural crest cells (NCC). Thus, cells of the SHF and NCC interact through signaling pathways, resulting in lengthening of the outflow tract and in normal migration and patterning of NCC to populate endocardial cushions

that septate the conus and truncus atriosus, i.e. the outflow tract, into the pulmonary artery and ascending aorta (Jiang et al., '00; Yelbuz et al., 02; Waldo et al., '05; High et al., '09). Each cell population requires the other to form and septate the outflow tract region (Waldo et al., 05; High et al., 09). NCC arise from neuroepithelial cells at the crests of the cranial neural folds, then migrate out of the folds down to the outflow tract region of the heart passing in close proximity to SHF cells (Fig.4; Jiang et al., '00; Hutson and Kirby, '03). 5-HT is important for these processes because it acts as a signaling molecule regulating NCC migration, proliferation and differentiation. Therefore, a disruption of 5-HT concentrations, as is caused by SSRIs like Zoloft interferes with this signaling, which in turn, alters normal NCC differentiation, adversely affecting the SHF and resulting in the cardiac defects as described in detail above.

The vital role 5-HT plays in NCC development has been documented in the scientific literature. For example, physiological concentrations of 5-HT stimulate migration of isolated NCC in an in vitro assay, whereas migration was inhibited by treatment with 5-HT receptor antagonists (Moiseiwitsch and Lauder, 1995). Similarly, treatment of mouse embryos in embryo culture with an antagonist to the 5-HT2B receptor resulted in cardiac defects, inhibition of neural crest cell migration, and cell death in the crest cell population (Choi et al., '97). NCC express both the 5HT2B receptor and the 5-HT transporter, SERT, which provides an explanation for these observations (Choi et al., 1997; Hanson et al., 99). As noted previously, migration, proliferation, and differentiation of crest cells is essential for normal development of the conotruncal region of the heart. Therefore, any adverse effect on this cell population, as would occur with abnormal 5-HT concentrations produced by Zoloft, would result in outflow tract defects, including pulmonary stenosis, tetralogy of Fallot, transposition of the great vessels, and common truncus arteriosus.

In addition to interacting with the SHF, NCC are also directly involved in patterning the great arteries derived from the aortic arches. Inhibiting this regulation, as occurs from abnormal concentrations of 5-HT produced by Zoloft, can result in abnormalities of these vessels, as described above (Kirby and Waldo, '95; Waldo et al., '96; Kirby, M.L., et al. 1997).

B. Septating the heart chambers by differentiation of endocardial cushions

Another target for SSRIs like Zoloft on heart development is endocardial cushion formation. Septum formation necessary to separate the heart into four chambers and the outflow

tract into the pulmonary artery and the aorta, begins late in the 4th week of gestation and is completed by the 7th week . The key to normal septation are endocardial cushions that form in the region surrounding the atrioventricular canal and the outflow tract Fig.5). Initially, these cushions represent expansions of cardiac jelly (produced by myocardial cells) between the myocardium and endothelial lining of the heart tube. This expansion only occurs in the atrioventricular region and the outflow tract (Figs. 5-7). Cushion formation is induced by the myocardium and it is these cushions that will be essential for septation (Lockhart et al., '11; Sadler, '12).

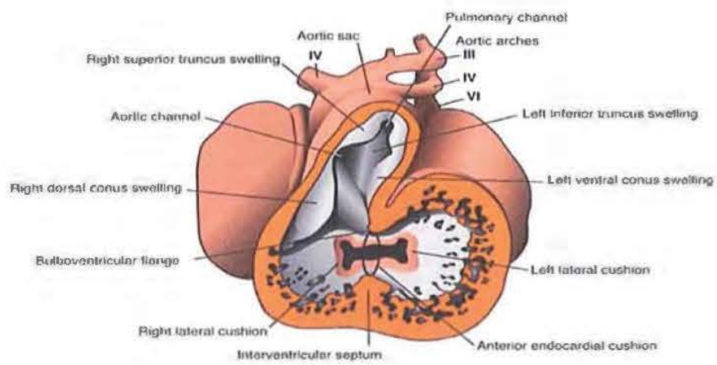

Figure 5. A. By 5 weeks endocardial cushions are present with 4 located around the atrioventricular canal (2 lateral, 1 anterior, and 1 posterior) and 4 in the outflow tract in the conus (right and left) and the truncus (right and left).

Following cushion formation by extracellular matrix deposition, additional signals from the myocardium, specifically in regions where the cushions develop, cause some of the endothelial cells lining the insides of the cushions to detach from their neighbors and transform into mesenchymal cells (Figs 6 and 7). Then, through cell migration, proliferation, and synthesis of additional extracellular matrix materials, these cells populate the cushions causing their continued growth (Figure 6: Eisenberg and Markwald, '95; Markwald et al., '96). A similar process occurs during cushion formation in the outflow tract (conus cordis and truncus arteriosus), but cells populating those cushions are derived from NCC (Fig. 4B), not endocardial cells (Jiang et al., '00; Hutson and Kirby, '03).

Positioning of the outflow tract and AV cushions may be dependent upon 5-HT signaling in the precise regions where cushions will form. For example, at initial stages of heart tube formation and looping, 5-HT uptake SERT sites appear throughout the myocardium. During the later stages of looping, these uptake sites become localized to the AV canal and outflow tract

(Figs. 6 and 7) where endocardial cushions will form, indicating that 5-HT signaling is important for positioning the outflow tract cushions and forming the mitral and tricuspid valves that are derived from these structures (Shuey et al., '90; Yavarone et al., 93).

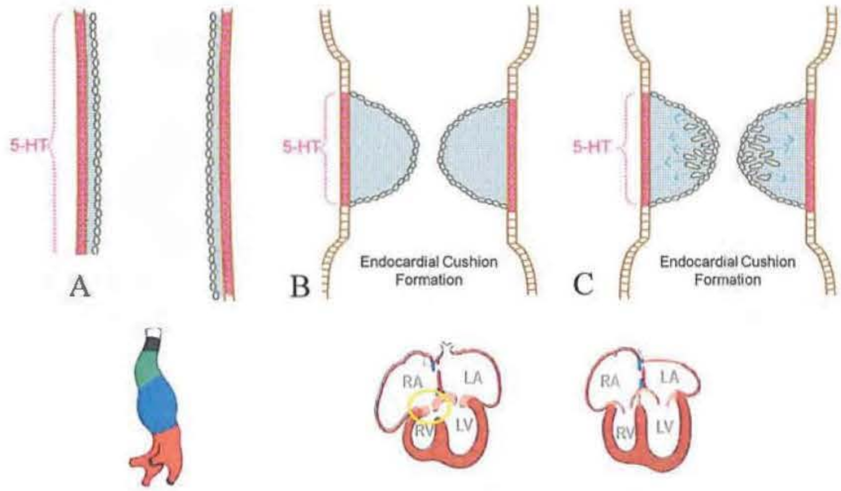

Figure 6. Formation of the endocardial cushions. **A.** Initially the heart tube consists of an external layer of cells called the myocardium, an inner layer called the endocardium, and a thin layer of extracellular matrix (blue). At this stage, all cells in the myocardium take up 5-HT. **B.** Cushions form in specific sites (atrioventricular canal and outflow tract) by increased synthesis of the extracellular matrix (blue). At this stage, 5-HT uptake by the myocardium is restricted to areas of cushion formation (pink). **C.** Eventually, cushions are populated by endocardial cells (in the atrioventricular canal) and by NCC (outflow tract) and these cells migrate toward the myocardial cells that take up 5-HT (pink) and 5-HT stimulates migration of these cells.

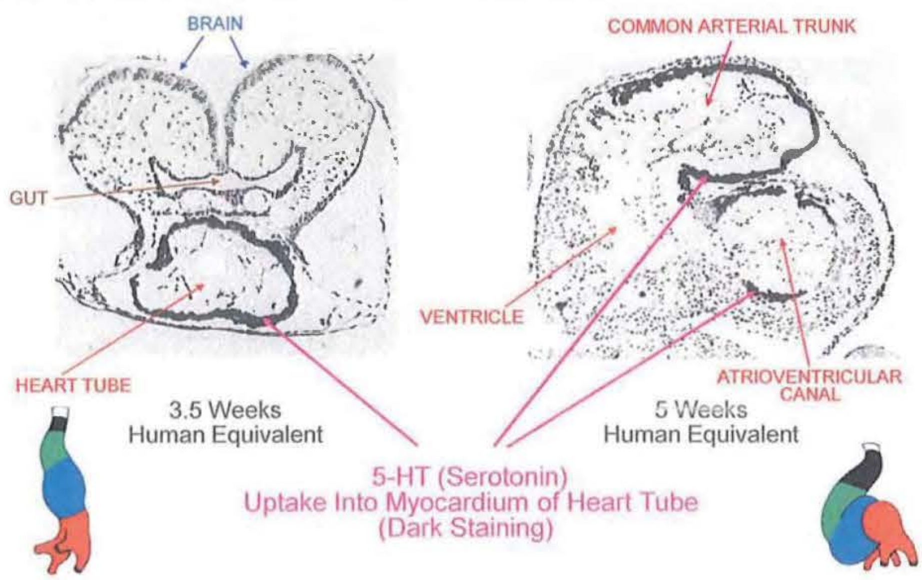

Figure 7. **A.** 5-HT (dark staining) uptake sites in the myocardium of a mouse heart at the tube stage of development (see Figure 6 A.). **B.** 5-HT uptake sites (dark staining) in the myocardium at a more advanced stage of development when endocardial cushions are forming in the atrioventicular canal and outflow tract (see Figure 6 C.).

As mentioned previously, endocardial cushions in the outflow tract depend upon NCC for their development. NCC originate from neuroepithelial cells at the edges (crests) of the neural folds as these folds elevate and fuse to form the neural tube. Cardiac neural crest cells form in the hindbrain region of the embryo in the 4^{th} week and migrate ventrally toward the heart along three of the major branches (numbers III, IV, and VI) called the aortic arches, that connect the outflow tract with the dorsal aorta (Fig. 4 B) By the end of the 4^{th} week, crest cells reach the heart to populate endocardial cushions in both regions of the outflow tract (called the conus cordis) and truncus arteriosus (Fig. 5; Jiang et al., '00; Hutson and Kirby, '03).

Four endocardial cushions form around the atrioventricular canal: two lateral, an anterior, and a posterior cushion (Fig.5). The anterior and posterior grow toward each other and fuse to separate the single atrioventricular canal into the right and left atrioventricular canals by the end of the 5^{th} week (Fig. 5). In addition some of this cushion tissue proliferates to form the membranous portion of the interventricular septum, thereby completing formation of that septum. Later, the cushions surrounding both canals differentiate into the tricuspid valve on the right and the bicuspid (mitral) valve on the left.

In addition to its role in positioning the endocardial cushions, 5-HT signaling is involved in differentiation of the cushions. 5-HT binding protein, which regulates localized concentrations of the neurotransmitter, is present in endocardial cushions during and after their formation (Yavarone et al., '93). Additionally, expression of the 5-HT2B receptor is localized to the myocardium before and after looping (Choi et al., '97). This receptor is coupled to the *ras* pathway that regulates cell proliferation (among other cellular phenomena; Lauder et al., '00). Proliferation and migration of endocardial cells in the AV canal cushions and NCC in the outflow tract cushions is essential for normal cushion differentiation. In the AV cushions, enodocardial cells break free from their neighbors and migrate toward myocardial cells that have taken up 5-HT (Figs. 6 and 7; Eisenberg and Markwald, '95; Markwald et al., '96). Because 5-HT signaling has been shown to stimulate cell migration and proliferation in cardiac cells, it is likely that such signaling plays a key role in this process (Yavarone et al., '93; Choi et al., '97). It has also been shown that stimulation of migration in cushion mesenchyme cells by 5-HT was concentration dependent, such that concentrations that were too high or too low inhibited migration (Yavarone et al., '93). Thus, if 5-HT signaling is disrupted by abnormal concentrations of 5-HT as is caused by SSRIs like Zoloft, malformations of the AV valves would occur,

including mitral insufficiency, tricuspid atresia, Ebstein's anomaly, and VSDs. Thus, the specificity of the localization of 5-HT uptake SERT sites and the presence of the 5-HT2B receptor and 5-HT binding protein suggest a critical role for serotonin in myocardial cell proliferation and differentiation and in endocardial cushion differentiation (For a discussion of the effects on NCC and resulting defects, see Section **A** above).

C. Promoting growth and differentiation of myocardial cells

As discussed above, uptake sites for 5-HT are present in myocardial cells that form the muscular tissue of the heart (Shuey et al., '90; Yavarone et al., '93). These cells also express the 5-HT transporter, SERT and the 5-HT2B receptor (Choi et al., '97: Sari and Zhou, '03). Studies have shown that 5-HT acts as a signaling molecule that increases proliferation in myocardial cells, whereas SSRIs like Zoloft inhibit their proliferation (Yavarone et al., 93; Sari and Zhou, '03). Furthermore, knocking out the 5-HT2B receptor causes neonatal death in mice due to severe ventricular hypoplasia caused by decreased proliferation of cardiac myocytes (Nebigil, et al., '00). Thus, the scientific literature indicates that heart defects such as hypoplastic left heart can be caused by SSRIs like Zoloft.

Table 1. Summary of SSRI Targeted Tissues and the Resulting Heart Defects

Target Tissue	Cell Process	Normal Effect	Birth Defects
Primary* Heart Field (Days 16-18)	Establishment of laterality and patterning	Formation of the four chambered heart	DORV, TGA, l-TGA, ASD VSD, atrial isomerism ventricular inversion dextrocardia
Heart Tube* (Days 22-28)	Genetic signaling cascade for normal looping	Looping	dextrocardia
AVC* Endocardial Cushions (Days 26-35)	Cushion formation: cell proliferation and migration	Division of the AVC into left and right channels; Formation of the Mitral and tricuspid valves and the IVS	VSD, mitral and tricuspid valve defects (mitral insufficiency tricuspid atresia); positioning and leaflet defects
Secondary* Heart Field (Days 22-28)	Splanchnic mesoderm ventral to the pharynx and signaling from neural crest cells	Lengthening and partitioning the outflow tract into aortic and pulmonary channels	Tetralogy of Fallot TGA, Pulmonary atresia and stenosis
Outflow * Tract (Conotruncus) (Days 36-49)	Neural crest cell migration, proliferation and viability	Formation of the conotruncal cushions for division of the outflow tract	Common truncus arteriosus and other outflow tract defects

Aortic * Arches (Days 22-42)	Neural crest cell migration, proliferation and viability	Patterning the arches into the great arteries	Anomalous right pulmonary artery; IAA Type B

*Serotonin (5HT) can affect each of the target tissues.

Days give an approximate estimation of periods of vulnerability and are calculated from the time of fertilization.

Atrioventricular canal (AVC); Interventricular septum (IVS); Double outlet right ventricle (DORV); Transposition of the great arteries (TGA); left transposition of the great arteries (l-TGA); Atrial septal defect (ASD); Ventricular septal defect (VSD); Interrupted aortic arch (IAA).

VII. Craniofacial Development and SSRI (Zoloft)-induced Malformations

As documented in section **V**, SSRIs like Zoloft can cause cleft lip and/or palate at the time the body axes are being patterned. These defects occur when the drug interferes with the signaling pathway involving 5-HT that establishes the L-R sides of the body, which in turn, affects midline structures, including the palate. In addition to this mechanism, SSRIs including Zoloft can cause cleft lip and palate by acting directly on cells and processes essential to normal lip and palate development. Thus, there are 3 targets for the teratogenic effects of these drugs acting directly on the lip and palate, including: 1) Neural crest cells (NCC) that form the nasal and palatal processes; 2) Epithelial-mesenchymal interactions essential for growth and differentiation of the nasal and palatal processes; and 3) Palatal shelf elevation and re-orientation.

A. Normal Lip and Palate Development

A key phenomenon in facial development occurs during the 3rd week of embryogenesis when NCC migrate from cranial neural folds into the facial region (Fig. 8A). Here, these cells proliferate and form the facial prominences (swellings), that include the frontonasal, maxillary, and mandibular prominences (Fig. 8B). Soon thereafter, ectodermal thickenings appear bilaterally on the surface of the frontonasal prominence to form the nasal placodes (Figs. 8B and C). These placodes then invaginate to form the nasal pits and ultimately the nares (openings) in the nose. As the placodes invaginate, they create paired swellings on each side of the face called the medial and lateral nasal processes (Figs. 8C and D). With continued growth of these nasal processes as well as the maxillary prominences during the 6th week of development, a line of fusion forms between the medial nasal prominences and maxillary prominences on each side of the upper lip region (Figs. 8D-F). Subsequently, in the 7th week, the maxillary prominence on